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# *Indian Standard*

# METHODS FOR DETECTION AND ESTIMATION OF DAMAGE IN COTTON FIBRES DUE TO MICRO-ORGANISMS

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# *Indian Standard*

# METHODS FOR DETECTION AND ESTIMATION OF DAMAGE IN COTTON FIBRES DUE TO MICRO-ORGANISMS

## 0. FOREWORD

**0.1** This Indian Standard was adopted by the Indian Standards Institution on 6 December 1964, after the draft finalized by the Textile Chemistry Sectional Committee had been approved by the Textile Division Council.

**0.2** Cotton fibres are liable to be attacked and damaged by micro-organisms, such as bacteria and fungi at three stages (i) during cultivation as cotton bolls on plants, (ii) after harvest as *KAPAS* in the farm storage, and (iii) as lint during transit and storage in godown. The deterioration is promoted by climatic conditions prevalent in tropical and subtropical countries. Formulation of standard methods for detection and estimation of damage in cotton fibres due to micro-organisms, therefore, needs no emphasis.

**0.3** Moisture is essential for the development and growth of micro-organisms, the amount of moisture required being dependent upon the type of organisms. Thus, fungi usually develop when the relative humidity of the environment approaches about 75 percent and the growth is rapid when the relative humidity is above 85 percent. Bacteria, on the other hand, are active only when the substrate on which they are present is itself wet.

**0.3.1** During cultivation the cotton plants and particularly the cotton bolls suffer microbial attack by micro-organisms from soil or environment. The extent of damage is governed by the micro-climate and the type of plant.

**0.4** Fungi may appear as fine downy growth or as dark spots or stains causing discolouration of fibres and forming fibrelocks in cotton bolls. Mould growth may affect bunch of fibres in limited and isolated regions with no tendering in between two such regions. But if the fibres had been in contact with soil or contaminated with it and then exposed to dampness, the entire fibre or whole of fibrelocks may show uniform tendering.

**0.5** The degree of growth of micro-organisms as well as their tendering effect may differ widely from fibre to fibre and within a fibre itself. It is, therefore, not necessary to submit the results of tests prescribed in this standard to statistical analysis.

**0.6** In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

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*Rules for rounding off numerical values ( *revised* ).

## 1. SCOPE

**1.1** This standard prescribes methods for the detection and estimation of damage in cotton fibres due to micro-organisms.

**1.1.1** The methods are applicable to cotton fibres in boll stage and also during storage and transit from the farm to the industry.

## 2. SAMPLING

**2.1** Samples for test shall be selected so as to be representative of the lot.

**2.2** The samples shall be first visually examined for the presence of mould growth visible as isolated dark portions, stains or discolouration in fibres or tufts of fibres. Such fibre lots shall be separated as test sample.

**2.2.1** In the absence of any indicative visible mildew symptoms, the samples shall be examined under ultraviolet light for fluorescence. Those fibres or tufts of fibres that show fluorescence shall be separated as test sample.

## 3. TEST SPECIMENS AND CONTROL SPECIMENS

**3.1** From the test sample ( *see* **2.2** and **2.2.1** ) draw at random 15 specimens each weighing about 5 g; these shall constitute the test specimens for the purpose of **5.2.1, 5.2.2, 5.2.4, 5.3.1, 5.3.2** and **6.1.1**.

**3.2** From that portion of the sample from which test sample ( *see* **2.2** and **2.2.1** ) has been separated, draw at random six specimens each weighing about 5 g; these shall constitute the control specimens for the purpose of **5.2.3, 5.2.5** and **6.1.3**.

## 4. REAGENTS

**4.0 Quality of Reagents** — Unless specified otherwise, pure chemicals shall be employed in tests and distilled water ( *see* IS : 1070-1960* ) shall be used where the use of water or distilled water as a reagent is intended.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

**4.1 Balloon Test Reagent** — prepared by dissolving 15 g of caustic soda in 85 ml of water and mixing equal parts of this solution and carbon disulphide.

**4.2 Lactophenol Solution** — prepared by dissolving 100 g of phenol in 100 ml of water and adding to the solution 100 ml each of glycerine and lactic acid.

**4.3 Cotton Blue Solution** — prepared by making a saturated solution (about 25 ml) of cotton blue (Colour Index No. 51190) in 95 percent alcohol and adding to 10 ml of this solution 10 ml of glycerine and 80 ml of water.

**4.4 Cotton Blue-Lactophenol Solution** — prepared by mixing equal volumes of cotton blue and lactophenol solutions.

**4.5 Congo Red Solution** — prepared by making a 2 percent solution of Congo red (Colour Index No. 22120) in water.

**4.6 Caustic Soda Solutions** — (i) prepared by dissolving 11 g of caustic soda in 89 ml of water, and (ii) prepared by dissolving 18 g of caustic soda in 82 ml of water.

## 5. DETECTION OF DAMAGE

**5.1 Apparatus** — For the purpose of this test, the following apparatus shall be used:

a) Electrical *p*H meter or Lovibond comparator, and

b) Microscope with a magnification range of 50 to 900.

### 5.2 Procedure

**5.2.1** Draw about one gram of the fibres from one of the specimens drawn as in **3.1**, place it in a clean, dry, wide-mouthed glass-stoppered bottle (50 ml capacity) and leave it at room temperature (temperature range 25°-28°C) for three hours. Remove the stopper and smell the contents of the bottle, taking a deep breath.

NOTE — The presence of characteristic musty odour is indicative of the growth and attack of micro-organisms. If the test samples are damp, the odour will be dominant.

**5.2.2** From another test specimen drawn as in **3.1**, take about one gram of the fibres and cut them into small bits. Take a wide-mouthed conical flask and rinse it thoroughly with distilled water. Place the bits in the flask, add 10 ml of distilled water and boil for half an hour. Take the supernatant liquid and determine its *p*H value with the *p*H meter or Lovibond comparator, noting down the temperature of the supernatant liquid.

**5.2.3** Determine the *p*H value of the aqueous extract of the control specimen drawn as in **3.2**, following the procedure prescribed in **5.2.2**.

NOTE — With fungal attack the *p*H value generally shifts towards the acid side, whereas with bacterial attack it generally shifts towards the alkaline side.

**5.2.4** Take about 50 to 60 fibres from the test specimen drawn as in **3.1**, mount them parallel on a glass slide, cover them with a cover plate and treat them with balloon test reagent. Examine the fibres under microscope after 30 minutes.

**5.2.5** Treat similarly the fibres from a control specimen drawn as in **3.2** and examine them under microscope.

NOTE — Undamaged fibres will show formation of balloons or beads ( *see* Fig. 1 ) whereas damaged fibres will not show formation of any balloons or beads ( *see* Fig. 2 ).

**5.3 Detection of Fungal or Bacterial Damage**

**5.3.1** From four test specimens ( *see* **3.1** ) take few fibres from the stained portions, mount them on a glass slide and irrigate them with a few drops of lactophenol solution. Cover the fibres with a cover plate and examine them under microscope.

NOTE — The presence of spores, fungal fructifications and hyphæ indicate damage due to fungi ( *see* Fig. 3 ).

**5.3.2** Take a sufficient number of fibres from four test specimens ( *see* **3.1** ) and place them in a watch-glass. Treat the fibres with a few drops of cotton blue-lactophenol solution for 1 to 2 minutes. Remove the colour from the surface of the fibres by treating them with lactophenol solution. Mount these fibres on a number of glass slides in lactophenol and examine them under the microscope.

NOTE — A magnification of 50 to 500 should be used for detection of damage due to fungal attack and a magnification of 500 to 900 should be used for detection of damage due to bacterial attack.

**5.3.3** If, under a magnification of 50 to 500, the slides prepared as in **5.3.2** ( *see* Fig. 4, 5 and 6 ) show, as compared to undamaged fibre ( *see* Fig. 7 ),

a) incisions or cracks on the cuticle of the fibre, and/or

b) damage to primary and secondary walls of the fibre from the cuticle inwards, and/or

c) hyphæ within the lumen,

report the lot to have been damaged by fungal attack. If, under a magnification of 500 to 900, the slides prepared as in **5.3.2** ( *see* Fig. 8 and 9, *and* Note 1 ) show, as compared to undamaged fibre ( *see* Fig. 7 ) indentations or serrations of the fibres from the cuticle inwards, that is towards

---

*Specification for water, distilled quality ( *revised* ).

the lumen, report the lot to have been damaged by bacterial attack ( *see* Note 2 ).

NOTE 1 — The extent of indentations or serrations is a good indication of the intensity of bacterial damage. Though in the case of fungal attack the primary and the secondary walls of the fibres are damaged, in the case of bacterial attack, the secondary wall and the primary wall immediately next to it do not usually indicate damage except in the case of highly affected fibres.

NOTE 2 — The larger the number of incisions or cracks on the cuticle of the fibres, the larger the quantity of hyphae within the lumen, and the greater the degree of damage of the primary and the secondary walls of the fibres from the cuticle inwards, the greater the damage by fungi.

## 6. ESTIMATION OF DAMAGE

### 6.1 Degree of Damage ( Damage Count Test )

**6.1.1** *Procedure* — Take about 0·1 g of fibres from four test specimens drawn as in **3.1** and place them in 50-ml beaker. Immerse the fibres in 11 percent caustic soda solution [ *see* **4.6** (i)] for three minutes and wash them thoroughly with distilled water. Treat the fibres with Congo red solution for 10 minutes. Rinse the fibres with distilled water to remove the excess of stain. Mount the stained fibres on glass slides and treat them with 18 percent caustic soda solution [ *see* **4.6** (ii) ] for one minute. Cover the fibres with cover plates and examine them under the microscope with a magnification of 200. Examine five slides and about 500 fibres.

NOTE 1 — Damaged fibres will show irregular stain patches, spiral staining and cracks. Severe bacterial damage is indicated by fluffy or fuzzy appearance of the fibres ( *see* Fig. 10, 11, 12 and 13 ).

NOTE 2 — Undamaged fibres will show three types of stains depending upon the maturity of the fibres. Immature fibres will show convolutions and pink stains. Half-mature fibres will become faintly red and highly mature fibres will show rupture due to swelling and reveal regular red stain spirals, which should not be mistaken for damaged fibres.

**6.1.2** Count separately the number of damaged and undamaged fibres on the slides. Calculate the damage count of the fibres as follows:

$$\text{Damage count} = \frac{a}{a+b} \times 100$$

where

$a$ = number of damaged fibres, and
$b$ = number of undamaged fibres.

**6.1.3** Repeat the procedure prescribed in **6.1.1** and **6.1.2** to determine the damage count of fibres taken from four control specimens drawn as in **3.2**.

**6.1.4** Compare the damage count values obtained as in **6.1.2** and **6.1.3**.

NOTE — Control specimens should have the lower damage count values than the test specimen.

FIG. 1 UNDAMAGED COTTON FIBRE SHOWING BALLOON FORMATION

FIG. 2 DAMAGED COTTON FIBRE SHOWING NO BALLOON FORMATION

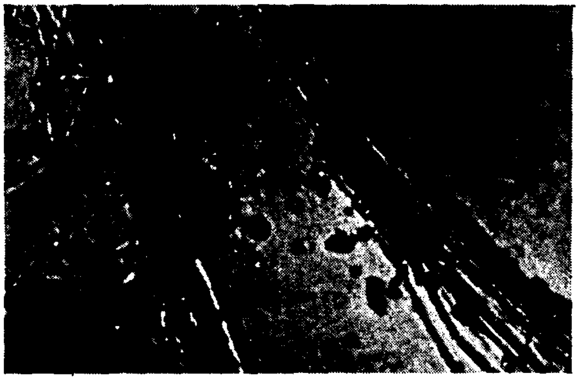

FIG. 3 COTTON FIBRE SHOWING FUNGAL GROWTH ( HYPHAE AND SPORES )

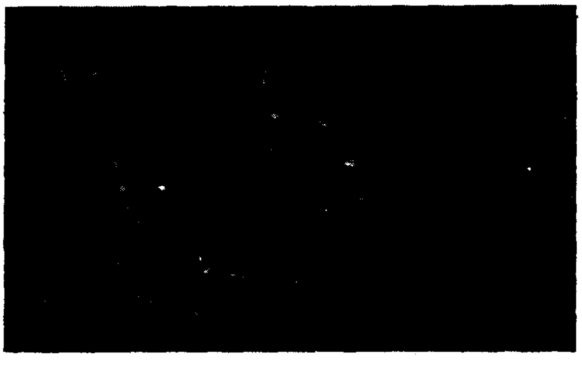

FIG. 4 COTTON FIBRE SHOWING INCISIONS OR CRACKS ON ITS CUTICLE

FIG. 5 COTTON FIBRE SHOWING DAMAGE TO ITS PRIMARY AND SECONDARY WALLS FROM THE CUTICLE INWARDS

FIG. 6 COTTON FIBRE SHOWING FUNGAL INFECTION — HYPHAE AND SPORES INSIDE AND LUMEN

FIG. 7 UNDAMAGED COTTON FIBRE

FIG. 8 COTTON FIBRE SHOWING MODERATE BACTERIAL DAMAGE

FIG. 9 COTTON FIBRE SHOWING SEVERE BACTERIAL DAMAGE

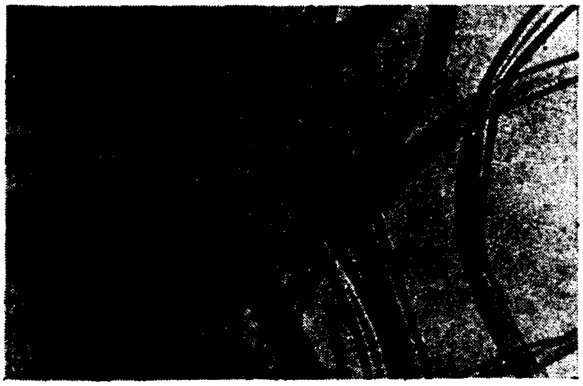

FIG. 10 UNDAMAGED COTTON FIBRE (AFTER CONGO-RED TEST)

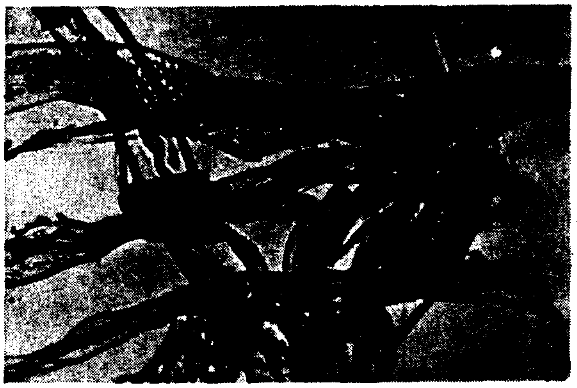

FIG. 11 COTTON FIBRE SHOWING MODERATE FUNGAL DAMAGE

FIG. 12 COTTON FIBRE SHOWING SEVERE FUNGAL DAMAGE

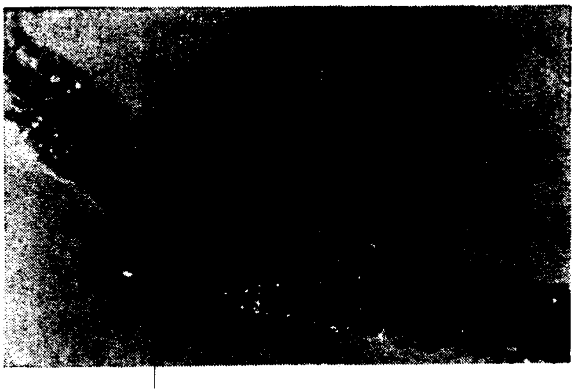

FIG. 13 COTTON FIBRE SHOWING SEVERE BACTERIAL DAMAGE

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